10/532447

JC20 Rec'd PCT/PTO 22 APR 2005

3,5,3'-TRIIODOTHYRONINE SULFATE AS THYROMIMETIC AGENT AND PHARMACEUTICAL FORMULATIONS THEREOF"

FIELD OF THE INVENTION

The present invention regards the use of 3,5,3'-triiodothyronine sulfate, usually named triiodothyronine sulfate or $\mathbf{T_3}$ sulfate or even better $\mathbf{T_3S}$, as an active principle, alone or in combination with thyroxine, in the treatment of pathologies due to organic deficiency of 3,5,3'-triiodothyronine. Accordingly, the same is usable for the preparation of thyromimetic pharmaceutical compositions.

BACKGROUND OF THE INVENTION

A number of iodothyronines are present in blood, which are directly produced by thyroid gland or are the result of peripheral metabolism of other iodothyronines. Among them, 3,5,3'-triiodothyronine (acronym $\mathbf{T_3}$) is deemed to be the biological active form of thyroid hormone (**TH**), because it has shown high affinity for the specific receptors of the same and is normally present in serum at a concentration sufficient for the activation of said receptors.

The main secretion product of thyroid gland in the healthy adult is thyroxine, commonly designated with the acronym $\mathbf{T_4}$. It is peripherically converted to its biologically active form, $\mathbf{T_3}$ (Ref.1), through enzymatic removal of an iodine atom from the external aromatic ring of the molecule by both type I and type II 5'-iodothyronine monodeiodinases (**type I MD** and **type II MD**, respectively). This metabolic pathway is the main mechanism of endogenous production of $\mathbf{T_3}$; on consequence, $\mathbf{T_4}$ can properly be considered a pro-hormone. On the other hand, a minor part of

T_3 is also directly secreted by thyroid. On average, the amount of **T_4** produced in an adult being of 70 Kg weight every day amounts to 100 μg, while the total production of **T_3** amounts to around 25 μg. 4-8 μg of **T_3** out of said 25 μg are directly secreted by thyroid and the remaining ones derive from the peripheral conversion of **T_4**.

T_3 undergoes two different metabolic pathways. The main metabolic pathway consists in the partial deiodination of the inner aromatic ring by type III 5-iodothyronine monodeiodinase (**type III MD**) to give 3,3'-diiodothyronine, which is biologically non-active and is further metabolized through deiodination or sulfoconjugation. The other metabolic pathway regards around 20% of the total amount of **T_3** produced by the body and brings on sulfoconjugation of **T_3** to give **T_3S**, which is not able to bond to the thyroid hormones (Ref.2), thus resulting biologically non-active (Ref.3).

Contrary to what happens with **T_3**, **T_3S** is not deiodinated by **type III MD**. Rather, it resulted to be an excellent substrate for **type I MD** (Ref.4), which converts it very quickly into 3,3'-diiodothyronine sulphate. On consequence it has been widespread common knowledge that, in the healthy adult being, sulfoconjugation of **T_3** to give **T_3S** represents a way for speeding up the catabolism of **T_3**, so facilitating its biliary and urinary excretion. Actually, it was found that serum levels of **T_3S**, physiologically low in the health adult, are higher when **type I MD** activity is reduced.

Yet, it has also unespectedly been found that, just in some body districts and organs, sulfatases exist which, under particular physiological conditions and situations, are able to convert again **T_3S** into its active form **T_3** (Ref's.7-9).

Such enzymes have been described in the intestinal microflora as well

as in body tissues like liver, kidneys and nervous central system (Ref.10).

Recently, it has been found that endogenous **T_3S** levels in serum are quite high during intrauterine life and as such are kept by the body, i.e. higher than the ones normally found in the adult being, at least until the forth month of postnatal life (Ref.11). Considering the essential role played by thyroid hormones during growth, in particular as far as nervous central system functions are involved, suppositions have been made about the possibility that, in this tissue, **T_3S** may also possibly be used by the body as an occasional source of **T_3**, if and when needed, during the first period of life. Studies performed on autoptic specimens of human nervous cerebral tissue post-mortem showed that the amount of **T_3** in the same results limited by **type III MD** (Ref.12). While this enzyme does not attack **T_3S**, it has been surmised that **T_3S** may exceptionally represent an alternative endogenous source of **T_3** hormone in those tissues which contain sulfatases able to reconvert **T_3S** into its active form, just in case a particular need of the hormone arises in said tissues (Ref's.8, 13).

Further studies have been performed to ascertain the effective role played by **T_3S** during production and metabolism of thyroid hormones. Said studies have recently demonstrated that it shows thyromimetic effects in hypothyroid rats (Ref.10) as well as in euthyroid rats (Ref.14). In both cases **T_3S** has shown a potency of around one fifth that of **T_3**. Moreover both treatments with **T_3S** and with **T_3** produced a significant reduction of serum levels of thyreotropic hormone **(TSH)** in euthyroid rats, thus showing to possess similar capability in inhibiting its secretion. On the contrary, in the case of hypothyroid rats, **T_3S** showed a poor capability of inhibiting **TSH** secretion when compared to **T_3**. It is well known that **TSH** is a highly responsive indicator to the functional status of thyroid gland

and consents to detect the smallest alterations of its hormonal secretion. Actually, its levels are higher under conditions of reduced thyroid functionality, even in those conditions that are defined as sub-clinical, while they are reduced when an excess of thyroid hormones are present. Accordingly, **T_3S** seems unespectedly non-comparable to **T_3** as far as its capability of inhibition on formation of **TSH** is involved.

In conclusion, particularly in view of the latest studies, a clear and complete knowledge of the biological role played by **T_3**S has not yet been reached.

In fact its main, well-grounded and universally accepted, feature is its non-biological activity, i.e. it is a biologically inert metabolite of **T_3** (Ref's.2 and 3), and the sulfation pathway is regarded as a metabolic activator of **T_3** catabolism (Ref.5).

On the other hand, only in particular tissues and under exceptional critical conditions due to shortage of thyroid hormone in those tissues, it has been shown its potential as an endogenous local source of **T_3**.

As a result, today the skilled technician is still facing a complex, somewhat conflicting, situation, which highlits only some of the biological characteristics of the product and needs more exhaustive in depth studies.

In any case, none of the several documents forming the state-of-the-art discloses, shows or suggests the possibility of using this anomalous metabolite of **T_3** in therapy. No close prior-art document, either of experimental nature or substantially speculative, either taken alone or in combination with other related documents, suggests the use, or even the potential use of **T_3S** as a medicament, taken as such or

preferably in combination with other thyroid hormones or pro-hormones, like, for example $\mathbf{T_4}$. The fact that, only in some specific tissues of the body and under particular, peculiar circumstances, part of $\mathbf{T_3S}$ can be reconverted into $\mathbf{T_3}$ does not mean, nor implies, nor suggests that it is possible to generalize this feature to the whole organism through exogenous administration of the product. In particular, there is no suggestion that oral administration of the product, even in protected form according to known methods of the pharmaceutical technique, may render it bioavailable also because it is well known that in those districts where suitable sulfatases are not present the same is rapidly metabolized and excreted through the bile and urines.

SUMMARY OF THE INVENTION

It has now unespectedly been found, and this is one of the aspects of the present invention, that $\mathbf{T_3S}$, as such or in association with other thyroid hormones or pro-hormones, preferably $\mathbf{T_4}$, and properly formulated according to the desired application, is particularly useful as a medicament to be used in all those pathologies caused by insufficient production by the body of the needed quantities of active thyroid hormones, in particular $\mathbf{T_3}$.

DETAILED DESCRIPTION OF THE INVENTION

In fact, it has unespectedly been found that the administration of $\mathbf{T_3S}$, contrary to what known about its normal metabolism, allows to maintain steady levels of $\mathbf{T_3}$ in the body for long times (from 12 to 18 hrs) and that results particularly useful in those cases in which it is needed to supplement thyroid hormone in its most active form.

Particularly preferred in the therapy of hypothyroidism, and this is a main aspects of the present invention, is resulted the association of **T_3S** with **T_4**. The hormonal association which, in theory, should more accurately mime the normal thyroid secretion is represented by a combination of **T_4** with **T_3**. Actually, pharmaceutical compositions comprising both of said iodothyronines, formulated in proportions similar to the ones of the normal physiologic secretion, have already been tried and marketed. Unfortunately, the oral simultaneous administration of **T_4** with **T_3** was not able to reproduce the normal thyroid hormones serum levels, because of pharmacokinetics of **T_3**. In fact, **T_3** undergoes a very quick absorption and an equally quick elimination after oral administration; its elimination rate is about 20 times higher than the one of **T_4**. For this reason administration of **T_3** gives raise to a dangerous peak excess in hormone concentration, if compared to the normal physiologic levels, followed by a too much fast drop to sub-physiologic levels. On consequence, today most of the specialised physicians prefer using **T_4** alone, even if in this way production of **T_3** only depends on the periferic deiodination of **T_4**, because direct secretion of **T_3** by thyroid does not exists or is seriously insufficient.

On the contrary, the association of the invention avoids the above problems, because it has unespectedly been found that, for example, after oral administration, **T_3S** provides **T_3** serum levels that increase in a gradual way and keep steady for long periods of time, thus preventing the formation of too much high peaks.

Another unespected advantage deriving from the use of **T_3S** in the treatment of pathologies due to organic deficiency of **T_3** consists in its recently found systemic thyromimetic activity linked to a poor inhibition of

TSH secretion. This effect is particularly useful in the case of thyroidectomized patients suffering from thyroid carcinoma, when administration of T_4 must be suspended in view of carrying out total body scintigraphy. In such a case administration of T_3S instead of T_4 may solve patient's necessity, without interfering with the diagnostic examination.

Another further advantage of T_3S in the therapy of hypothyroidism regards its autolimitation capability. In fact, it is actively deiodinated by **type I MD**, which, on its part, is stimulated by thyroid hormones. In hypothyroid subjects **type I MD** activity is reduced; on consequence also T_3S elimination is slowed. As a matter of fact, its effect on the body results greater. On the contrary, in case of over administration, **type I MD** activity is increased, thus giving more T_3S elimination, i.e. limiting possible undesired collateral effects.

Last but not least, a further advantage of T_3S is represented by the fact that it is a metabolite normally present in the body, usually non-active, i.e. non-toxic. On consequence problems of hypersensitivity or intolerance following its administration are not reasonably predictable.

Accordingly, another main aspect of the present invention regards pharmaceutical formulations comprising T_3S as an active principle, as such or in combination with other thyroid hormones or pro-hormones. Particularly preferred are formulations comprising T_3S in association with T_4.

Said formulations differ in the dosage of the active principle or principles, or in the type of pharmaceutical form provided, depending on the desired administration kind. Moreover they can also contain useful additives like excipients, diluents, dissolvents, solvents, carriers, dyestuffs, flavourings, sweeteners commonly used in the pharmaceutical technology.

The preparation of specific pharmaceutical formulations in response to particular needs of administration is plainly comprised in the general technical field of the present invention.

EXPERIMENTAL SECTION

As an example, absolutely non-limiting for the skilled technician, **T_3S** may be administered for oral use at doses ranging from 5 to 1000 μg, preferably from 10 to 500 μg, more preferably from 25 to 250 μg.

Analogously, when in association with **T_4**, preferred doses range from 10 to 500 μg for **T_3S** and from 10 to 250 μg for **T_4**, more preferably from 25 to 250 μg for **T_3S** and from 25 to 200 μg for **T_4**.

Two representative formulations for oral administration, selected among the preferred ones, are hereinafter enclosed by way of an example. Obviously, said formulations have no limiting effect on the other possible variations, which may also comprise different types of administration, different doses or different components depending on the specific pharmacological application or the particular pathology.

Example A) – Oral formulation containing **T_3S**

T_3S	50	μg;
Calcium phosphate dibasic anhydrous	103.5	mg;
Mais starch	17.65	mg;
Microcrystalline cellulose	5	mg;
Sodium carboxymethylamide	5	mg;
Talc	5	mg;
Citric acid	2.8	mg;
Magnesium stearate	1	mg

Example B) – Oral formulation containing **T_3S** and **T_4**

T_3S	50	μg;
T_4 sodium salt	125	μg;
Calcium phosphate dibasic anhydrous	103.5	mg;
Mais starch	17.525	mg;
Microcrystalline cellulose	5	mg;
Sodium carboxymethylamide	5	mg;
Talc	5	mg;
Citric acid	2.8	mg;
Magnesium stearate	1	mg

In particular, when the association is taken into account, the formulations of the present invention will also possibly comprise individually formulated doses of **T_3S** and **T_4**, so that sequential administration is possible. In this case, one suitable kit is provided, which consents distinct administration of said active principles in ways that can differ from patient to patient, depending on the needed therapeutic application. In such a way, the specialized physician will have a wide choice of changing the prescription according to the actual need of the patient.

Just by way of an absolutely non-limitative example, in the case of oral administration, one package containing two individual blisters, which have different shape and/or color and/or different contents and/or doses, may suit the desired scope. Other possibilities exist and are easily available to the expert of the field.

The pharmaceutical compositions of the present invention are usable in the treatment of pathologies due to organic deficiency of

triiodothyronine (**T_3**), like, for example, original hypothyroidism from autoimmune thyroid affections, hormonal production defects, thyroidectomy, congenital hypothyroidism, as well as some disorders due to reduced activity of type I 5'-iodothyronine monodeiodinase (**type I MD**) which is induced, for example, by hypothyroidism, non thyroidal systemic illnesses, fast, selenium shortage and so on.

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